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Kinetic Measurements of Bone Mineral Metabolism The Use of ^{22}Na as a Tracer for Long-Term Bone Mineral Turnover Studies

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THE USE OF ^{22}NA AS A TRACER FOR LONG-TERM BONE MINERAL TURNOVER STUDIES

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ABSTRACT

Sodium-22 has been studied as a tracer for bone mineral metabolism in rats and dogs. When incorporated into bone during growth from birth to adulthood, the bone becomes uniformly tagged with ^{22}Na which is released through the metabolic turnover of the bone. The ^{22}Na which is not incorporated in the bone matrix is rapidly excreted within a few days when animals are fed high but nontoxic levels of NaCℓ. The ^{22}Na tracer can be used to measure bone mineral loss in animals during space flight and in research on bone disease.

This paper is based on work performed under NASA Contracts NAS 9-14248, NAS 9-15544, and PO-A-13044B(KT).

The animals involved in this study were maintained and used in accordance with the Animal Act of 1970 and the "Guide for the Care and Use of Laboratory Animals", prepared by the National Academy of Sciences, National Research Council.

INTRODUCTION

The elements calcium, phosphorus, oxygen, hydrogen carbon, and nitrogen make up more than 99% of human bone, but none of these elements has a long lived radioisotope which emit gamma rays which can be measured outside the body. Therefore, it is difficult to perform long-term bone mineral metabolism studies using an isotope of these elements. Sodium is a small (0.6%) component of bone but previously has not been recognized as a true bone mineral. It has been suggested that the location of Na in bone is on crystal surfaces rather than in the bone crystal itself (14). This may be true of Na which has been recently incorporated into the bone of mature skeletons but experiments described in this paper indicate that a relatively nonexchangeable fraction of Na in bone is contained within the bone crystal. Sodium-22 so incorporated into bone mineral is an excellent tracer for bone mineral loss and turnover studies. These experiments were conducted to develop a method of measuring bone mineral loss in animals during space flight, but may have application to other bone mineral metabolism studies.

SODIUM METABOLISM

Most of the Na in the body exists in extracellular body fluids. About 40% of the total Na is in bone, but nearly half of this is readily exchangeable (12). This leaves about 20% or about 20 grams of Na which is fixed in adult human bone. Studies have shown that Na is a small but constant component of bone mineral in both cortical and trabecular bone (1,5,8,9). The constant ratio of Na to Ca in bone is shown in Figure 1, which shows Ca and Na content of dry bone from autopsy and biopsy samples from 19 people. This group included both normal people and those in various stages of renal failure having various bone densities (9). The error bars represent uncertainty in a single measurement. The ratio of sodium to calcium is constant, indicating that the resorption of bone mineral results in the same fractional removal of both elements.

Another study which indicates that Na in bone remains fixed until the bone crystal is resorbed is that conducted by Burch (3). The uptake of ^{22}Na in bone was observed by whole body counting after the administration of 50 μCi to an adult human. Figure 2 shows the retention in bone after ^{22}Na has left the soft tissues. Biological half-lives of ^{22}Na in some parts of bone up to 3.5 years are evident and much longer half-lives are indicated. Since the ^{22}Na remained in the soft tissues and fluids only a few weeks, the amount of deposition into bone having a lower turnover rate was very small and the size of these low turnover rate bone pools cannot be determined from this experiment.

From these two experiments it appeared that if ^{22}Na were uniformly incorporated into the bone of animals from birth to adulthood, both the turnover rate and quantity of the various types of bone could be determined, providing information about long-term bone mineral kinetics. This method should also allow the determination of the effects of weightlessness during space travel on bone mineral metabolism.

Sodium-22 has a decay half-life of 2.6 years and emits high energy gamma rays of 0.51 and 1.28 MeV, which are easily measured by whole body counting

techniques. A sodium tracer has another advantage over other bone mineral tracers such as ^{47}Ca or ^{85}Sr in that the ^{22}Na released from bone during resorption is greatly diluted by the large amount of stable sodium in soft tissue fluids and only an insignificant amount is recycled into newly formed bone. As much as 100% of ^{47}Ca or ^{85}Sr tracer released from bone can be recycled back into newly formed bone which makes bone turnover rate studies difficult to interpret using these tracers (6).

THE USE OF ^{22}NA TO MEASURE BONE RESORPTION RATES IN RATS

Bone resorption rates can be greatly increased in young growing rats by placing them on a calcium-deficient diet (14). Resorption rates three to four times normal can be caused in younger rats, but as they grow older only small increases in bone resorption occur with calcium deficiency. In this experiment, rat bone was uniformly tagged with ^{22}Na. The rat was then placed on a calcium-deficient diet to cause increased bone resorption.

In order to obtain rats with ^{22}Na uniformly distributed in the bone, female rats were raised from their conception to about 90 days of age on a nutritionally adequate synthetic diet tagged with ^{22}Na. For comparison, another group of rats were raised from conception to 77 days of age on a diet tagged with ^{85}Sr. The reason for doing this was that the ^{85}Sr closely follows calcium metabolism, and as bone is resorbed the ^{85}Sr will be temporarily released but to some extent will be reabsorbed into new bone just as calcium. If this is true, ^{85}Sr will not be a good indicator of increased bone resorption. It is expected that ^{22}Na would be released upon bone resorption but would not be significantly reabsorbed into new bone; therefore, it would be a sensitive indicator of increased bone resorption.

The radioactive food contained 0.060 μCi per gram of food for the ^{22}Na studies and 0.014 μCi per gram of food for the ^{85}Sr studies. The average amount incorporated into bone of each rat at the end of the radioactive diet was about 0.14 μCi of ^{22}Na for the ^{22}Na studies and 1.68 μCi of ^{85}Sr. The resulting high whole body counting rates allowed short accurate measurements.

At 77 days of age the rats raised on the ^{85}Sr diet were placed on a non-radioactive diet. Half of the 12 rats ate a normal diet and the other half

ate a calcium-deficient diet. After allowing seven days for the radioactivity to clear the gastrointestinal tract, the rats fed ^{85}Sr were whole-body counted once each week between two 4-inch-thick by 9-3/8-inch diameter NaI(Tℓ) scintillation detectors. They were placed inside a tube which restricted their movement and allowed reproducible counts to be made. Initial counts before and after thorough bathing of the rats showed that there was no significant external contamination. Subsequent counting of bone, soft tissue, and skin separately showed that all the measured ^{85}Sr was in the bone. The whole-body counting results for the ^{85}Sr tagged rats are shown in Figure 3. Even though increased bone resorption occurred in those growing rats eating a calcium-deficient diet, the ^{85}Sr content remained the same as that of the calcium-adequate rats. This apparently occurred because the rats became very efficient in incorporating the ^{85}Sr back into newly formed bone. Therefore it appears that changes in ^{85}Sr levels in bone cannot be used as an indicator of bone resorption, especially in cases of calcium deficiency.

At 90 days of age half of the 12 rats raised on ^{22}Na were placed on a nonradioactive normal diet and the other half on a nonradioactive calcium-deficient diet. Most of the ^{22}Na was in the soft tissues and periodic whole body counts were not started until near the end of the soft tissue clearance. This took place with a biological half-time of 7 days. The first counts were made at six weeks after the end of the radioactive diet, at which time the soft tissue clearance was not quite complete. At eleven weeks the bone contained essentially all the ^{22}Na in the body and the ^{22}Na content in the calcium-deficient rats was only 40% of the rats on the normal diet (see Figure 4). This indicates that the bone resorption during the period of 90 to 170 days of age in the calcium-deficient rats was more than two times that of the rats on a normal diet (see Reference 6 for more details of this study in rats).

The slow normal loss of ^{22}Na from soft tissues and fluids in the body limits the usefulness of ^{22}Na as a bone mineral tracer. An experiment was conducted in rats to develop a method for rapidly removing the ^{22}Na not associated with bone. Rats were injected with ^{22}Na which was allowed to equilibrate for 8 days into the soft tissues with some incorporation into

bone. The rats were then allowed to eat food containing up to 16 times the usual amount of Na in a normal diet. Figure 5 shows that the retention half-time of the ^{22}Na in soft tissue can be reduced from six days for rats on a normal diet to 0.7 day for rats on a diet containing at least 4.5% Na. (The data for the 1.0% Na in the diet was supplied by D. Baylink, Veterans Administration Hospital, Tacoma, Washington.) This high-sodium diet caused a tremendous increase in the water intake by the rats, but no adverse effects were observed. Thus an animal can be grown to maturity on a ^{22}Na diet to uniformly tag all bone at which time one can clear the extraosseous ^{22}Na in about 5 days with a high-sodium diet. Figure 5 shows that the body burden of ^{22}Na actually leveled out before the end of the high-sodium diet. This leveling out was due to that ^{22}Na which had become deposited in bone and was being released at the normal bone turnover rate. For more details of this study, see Reference 7.

THE RETENTION OF ^{22}NA IN DOG BONE

Since the skeleton of a rat does not completely stop growing, the study of ^{22}Na as a tracer for bone mineral metabolism was transferred to dogs which do acquire a mature skeleton of constant size as the dog reaches adult age.

Six female beagle puppies were selected for the study and at ten to twelve weeks of age the puppies were started on a special diet containing ^{22}Na. The food consisted of a low-sodium dog food (0.01% Na)* into which was thoroughly mixed a solution of NaCℓ containing a fixed ratio of ^{22}Na to stable Na. This brought the stable Na content of the food up to 0.12% and the ^{22}Na content in the food to 0.5 μCi per gram of Na or 0.28 μCi per pound of food. Assuming that a 10 kg beagle dog will have about 3 grams of non-exchangeable Na in the skeleton and allowing for the decay of the ^{22}Na during growth, the skeletal burden at eighteen months of age should be 1.2 μCi.

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*Prescription Diet h/d, a low sodium dietary food manufactured by Hill Packing Company, Topeka, Kansas

Due to a reduction in funding of this experiment, two of the six dogs were removed from the experiment when they were 9 months old. At 18 months of age, the ^{22}Na was removed from the diet of the four remaining dogs. Thirty grams of NaCℓ were added daily to the food of each dog for 10 days. This high level of Na in the diet completely removed the ^{22}Na from the soft tissues and fluids of the body by dilution, leaving only that ^{22}Na bound in the bone mineral. The removal half-time of ^{22}Na from the extraosseous part of the body was about one day, and the high NaCℓ content in the diet did not appear to cause any ill effects or inhibit food intake.

The total body content of ^{22}Na was measured in each dog starting the day the ^{22}Na was removed from the food. A drawing of the whole body counter used is shown in Figure 6. The lucite box, specially designed for measuring radioactivity in beagle dogs, holds the dogs in a fixed, reproducible position (2). A shielded 10.2-cm-thick by 23.8-cm-diameter NaI(Tℓ) detector located 83 cm from the lucite box was used to measure the 0.51 MeV annihilation photons emitted from the ^{22}Na decay. A standard ^{22}Na source was measured each day that the dogs were measured. The dog count was expressed as a fraction of the standard source count which gave a relative count corrected for radioactive decay. The counts were then converted to a fraction of the body content determined in the first count.

On the ninth day after the end of the ^{22}Na diet, when most of the soft tissue ^{22}Na had been excreted, the dogs were bathed and most of their hair clipped off. About 5% of the initial body content was deposited on the skin and less than 1% incorporated in the hair. Further bathing of the dogs on following days did not change the total body count. All previous whole body counts were corrected for this external contamination.

The whole body counting data are shown in Figure 7. The rapid loss of ^{22}Na during the first few days is that which was excreted from the soft tissue compartments. The ^{22}Na deposited in bone is unaffected by the high levels of Na in the body because it is relatively unexchangeable. After the tenth day the whole body count represents the skeletal content of ^{22}Na, and the loss of ^{22}Na beyond that day is due to normal bone mineral resorption.

During the first 100 days the average skeletal loss was 20% for dogs 39F, 51F, and 52F. During the next 92 days, the loss was 10% for the same three dogs. Dog 49F was not included because it was on a special diet which will be described later. The average retention half-time for the three dogs was determined by least squares curve fitting for an exponential type curve for the first 100-day period and the following 92-day period and was found to be 300 and 500 days respectively. As the ^{22}Na leaves the more metabolically active bone the remaining ^{22}Na is in bone which has a slower turnover rate.

At the end of the ^{22}Na diet the ratio of ^{22}Na to stable Na in all parts of the body is the same. If the bone content of the four dogs shown in Figure 7 is extrapolated back to zero time after the end of the ^{22}Na diet, the average fraction for the nonexchangeable or very slowly exchangeable Na in the bone is 11.2% of the total body Na. This is significantly less than the 20% fraction that has been estimated for human bone (12). The average amount of ^{22}Na in the bone of the four dogs was determined to be 0.57 μCi, which is lower than predicted 1.2 μCi but this is due to the lower amount of nonexchangeable Na actually found in the dog bone compared to that estimated in human bone.

One of the dogs (38F) was removed from the experiment and from the ^{22}Na diet when 12 months old, because of reduced funding, and became available for whole body counting at the age of 18 months. The whole body counts on this dog are shown in Figure 8. The whole body counts on this dog are shown in Figure 8. Since the weight of the dogs was approximately the same at 12 months as it was at 18 months, the amount of ^{22}Na in the body at 12 months, corrected for decay, was about the same as that at 18 months. The retention of 5.8% in the skeleton of dog 38F was only half the 11.2% average for the four dogs which were fed ^{22}Na until 18 months old. The difference represents the amount of bone mineral turnover in dog 38F between 12 and 18 months and the added bone mineral of the other four dogs as their skeleton reached maturity between 12 and 18 months. Assuming that skeletal weight increases only 5% between 12 and 18 months in beagles (4), then 45% of the bone mineral of dog 38F was replaced during this period. The average percent

bone mineral turnover determined by the ^{22}Na loss for dogs 39F, 51F, and 52F between 18 and 24 months of age was 27%. The higher turnover rate between 12 and 18 months is in agreement with other studies (11) which show that skeletal maturity is not reached until 18 months. The retention half-time for dog 38F between 21 and 24 months of age is estimated to be 930 days.

INDUCED BONE RESORPTION STUDIES

The original plans for this experiment included the inducement of bone mineral loss in some of the dogs and measurement of the associated ^{22}Na loss. These plans included inducing loss in the leg through disuse osteoporosis by placing a dog's leg in a cast for two months and in the total body by inducing secondary hyperparathyroidism through a lean meat diet. Although much valuable information was obtained from the latter study, funding was terminated before the full effect of the secondary hyperparathyroidism could be observed.

Thirty-six days after the end of the ^{22}Na diet dog 49F was started on a diet of unsupplemented lean meat consisting of 50% beef heart and 50% liver. Previous studies have shown that this type of diet over a twelve-month period will cause a 20% loss of bone mineral in the vertebrae and a 13% loss in the long bones (11). In Figure 7, the retention curve for dog 49F shows a possible increased loss of ^{22}Na starting about four months after the start of the lean meat diet. Figure 9 shows the retention of dog 49F compared with the average retention of the other three dogs and shows more clearly the apparent increased loss of ^{22}Na starting at 150 days after the end of the ^{22}Na diet. Another one or two months on this diet would probably have shown this ^{22}Na loss much more conclusively.

DISCUSSION

The results of these studies provide additional insight on the association of sodium in bone. It appears that the fixed fraction in dogs is lower than that estimated for humans. Isotope dilution studies in humans show that about 27% of the sodium in the body is not exchangeable (10); however, these results were obtained after a 24-hour period for equilibration and the dif-

ference between the 27% for humans and 11% for dogs may be due to a semi-exchangeable pool which exchange between 1 and 8 days.

The results obtained in these studies indicate that the ^{22}Na uniformly grown into bone remains in the bone until it is resorbed. It is difficult to prove that the Na remains as long as calcium or phosphorus, but the retention data indicate that it does. If the study had been continued for a few years the size and the metabolic rate of the various bone pools in the body could have been determined. From the 6-month observation on the dogs it can be seen that there is a 20% fraction of the bone in which the mineral atoms have a mean residence time of 432 days ($t_{1/2\ B}$ = 300 days) and a 10% fraction which has a mean residence time of 721 days ($t_{1/2\ B}$ = 500 days). The data from dog 305 indicate that there is a 4% fraction which has a mean residence time of 1340 days ($t_{1/2\ B}$ = 3.7 years) and therefore more than half of the bone mineral of the adult dog has a mean residence time of at least 3.7 years and probably much more. The data from a similar study using ^{90}Sr show a higher retention of ^{90}Sr after the end of the ^{90}Sr diet but this is undoubtedly due to the recycling of the ^{90}Sr into newly formed bone which does not occur with ^{22}Na. A two- or three-year observation of dogs in this type of experiment would provide a rather complete picture of the size and metabolic rates of all bone pools of interest.

The uniform tagging of bone mineral with ^{22}Na and subsequent measurement of the retention as described in this paper should provide a good measurement of enhanced bone mineral loss in animals which may occur during space flight. The method should be considered for space flights which last for two or more months. These methods should also be useful in many animal research studies of bone disease.

The experimental procedure for a space flight experiment would be to raise several dogs from puppies to adult age on a ^{22}Na diet. About 6 weeks before the start of a space flight ^{22}Na would be removed from the diet and the non-bone ^{22}Na removed by high levels of NaCℓ in the diet. A retention slope would be established for each dog. Half of the dogs would be placed on the space flight and the other half would remain on earth as controls.

After the space flight the dogs would be measured in the whole body counter and compared to the control dogs to determine any enhanced bone mineral loss or turnover rate. Sodium-22 in individual bones could be measured to determine losses in specific parts of the skeleton.

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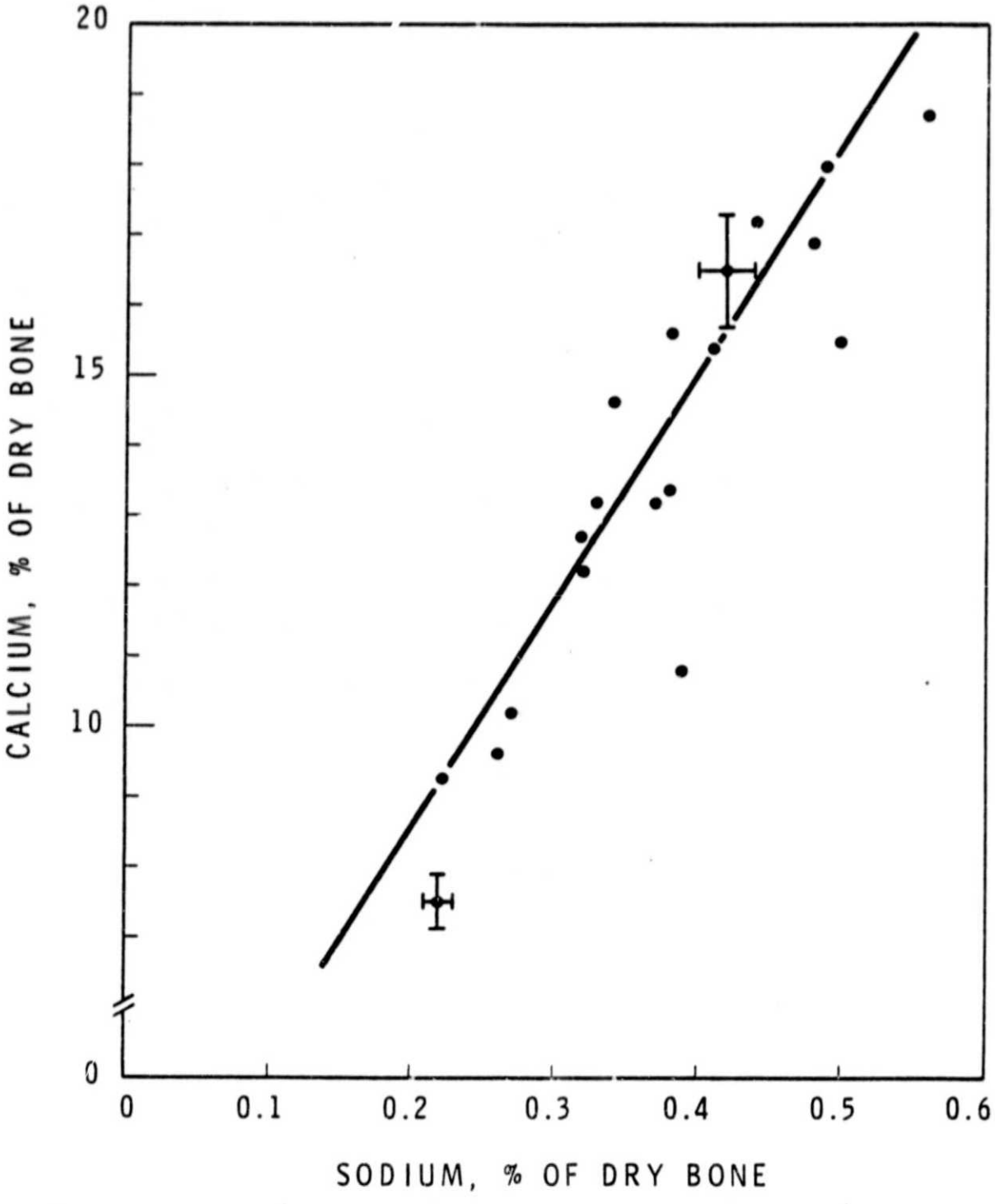

FIGURE 1. Calcium Versus Sodium Content of Human Bone

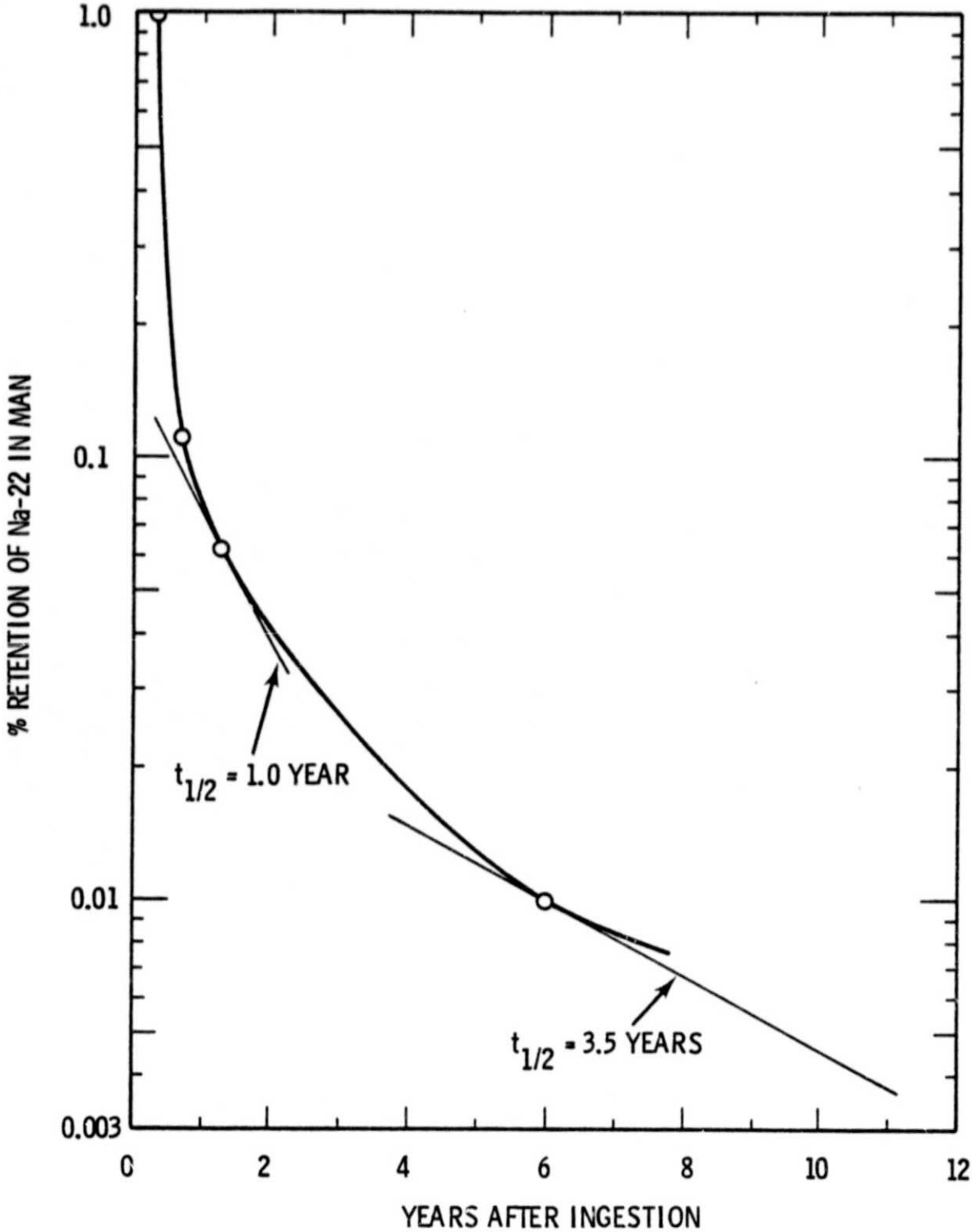

FIGURE 2. Retention of ^{22}Na in Adult Human Starting 100 Days After Administration of 50 μCi (Data Corrected for Radioactive Decay)

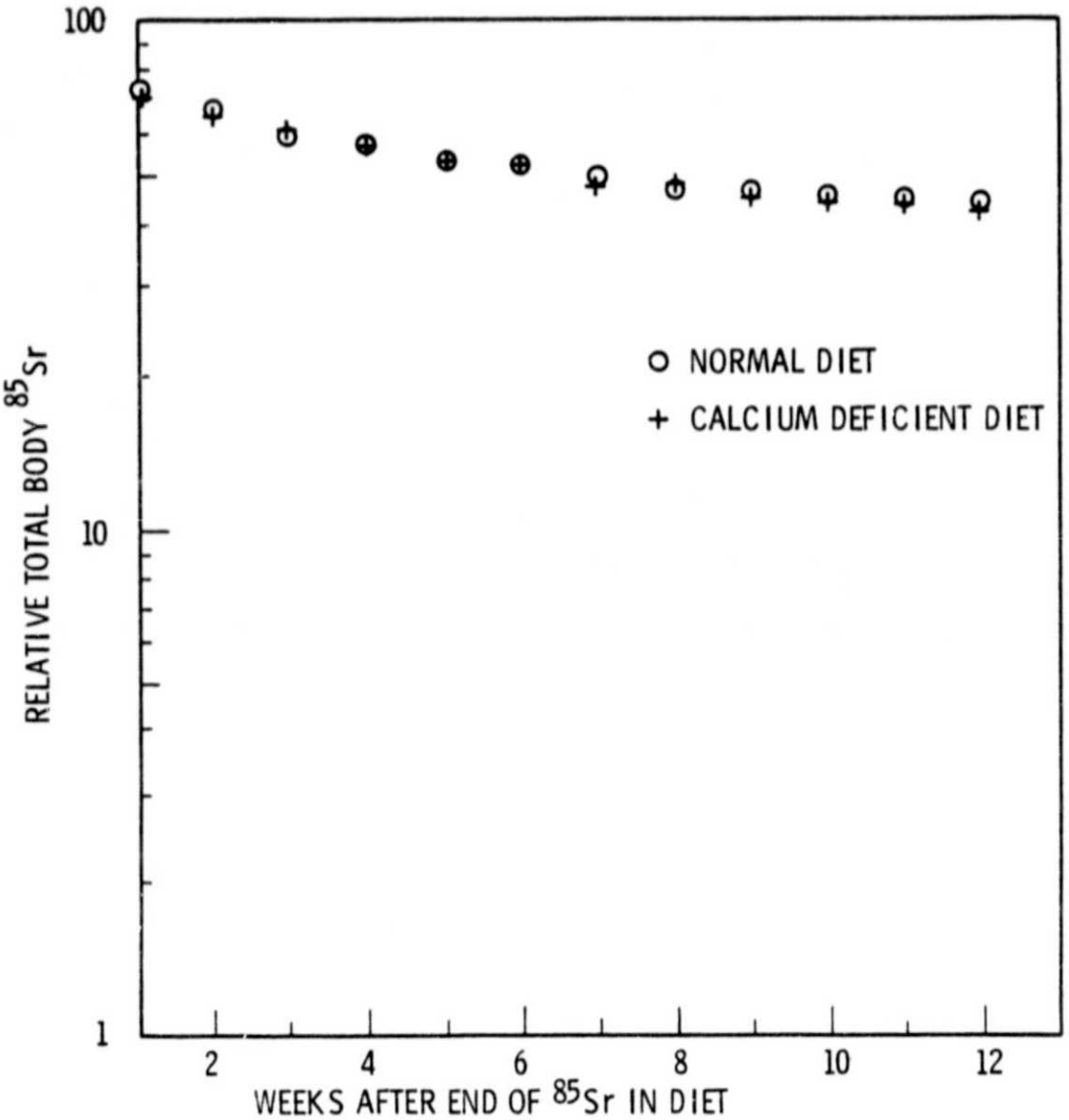

FIGURE 3. Comparison of ^{85}Sr Levels in Calcium Deficient and Normal Rats Starting with 14-Week-Old Rats

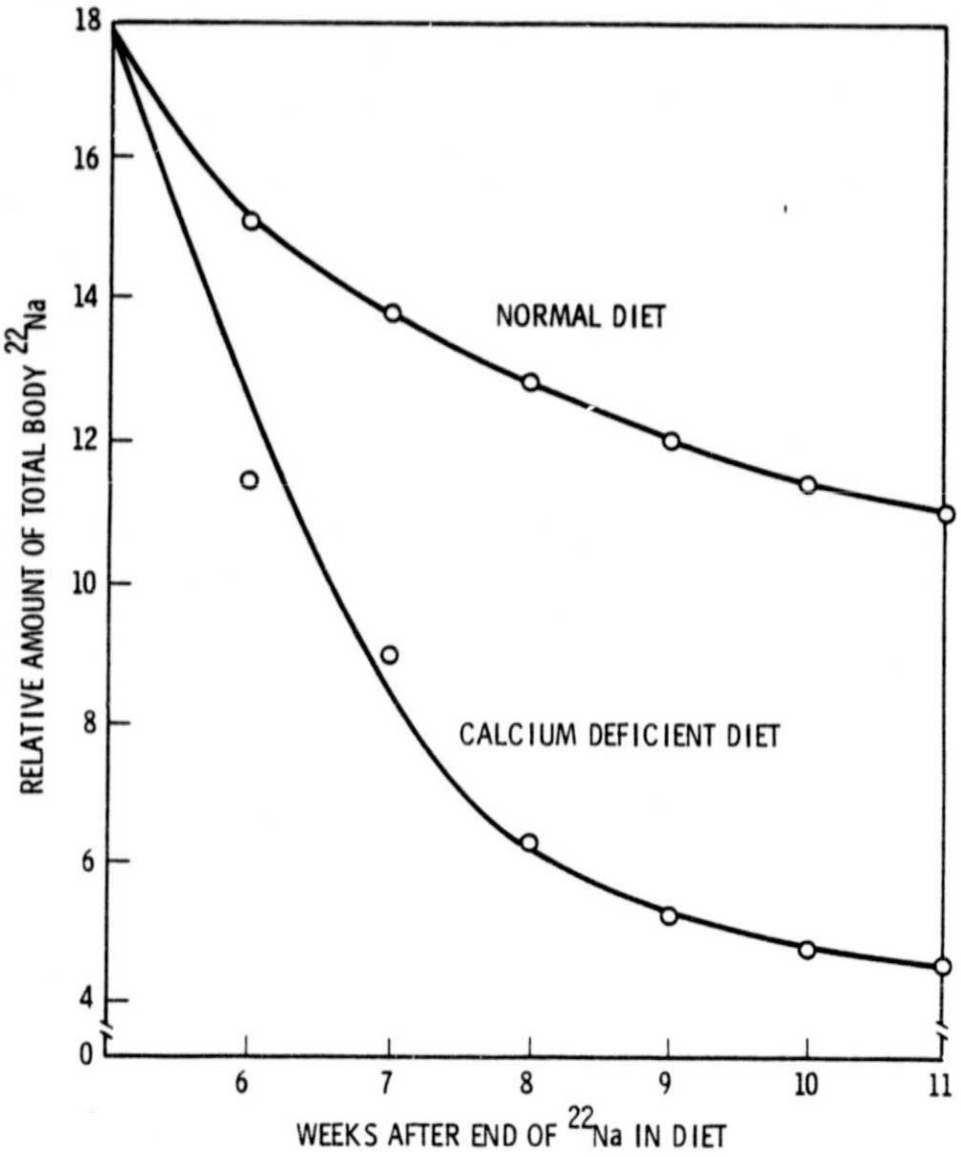

FIGURE 4. Comparison of ^{22}Na Levels in Calcium Deficient and Normal Rats Starting with 18-Week-Old Rats

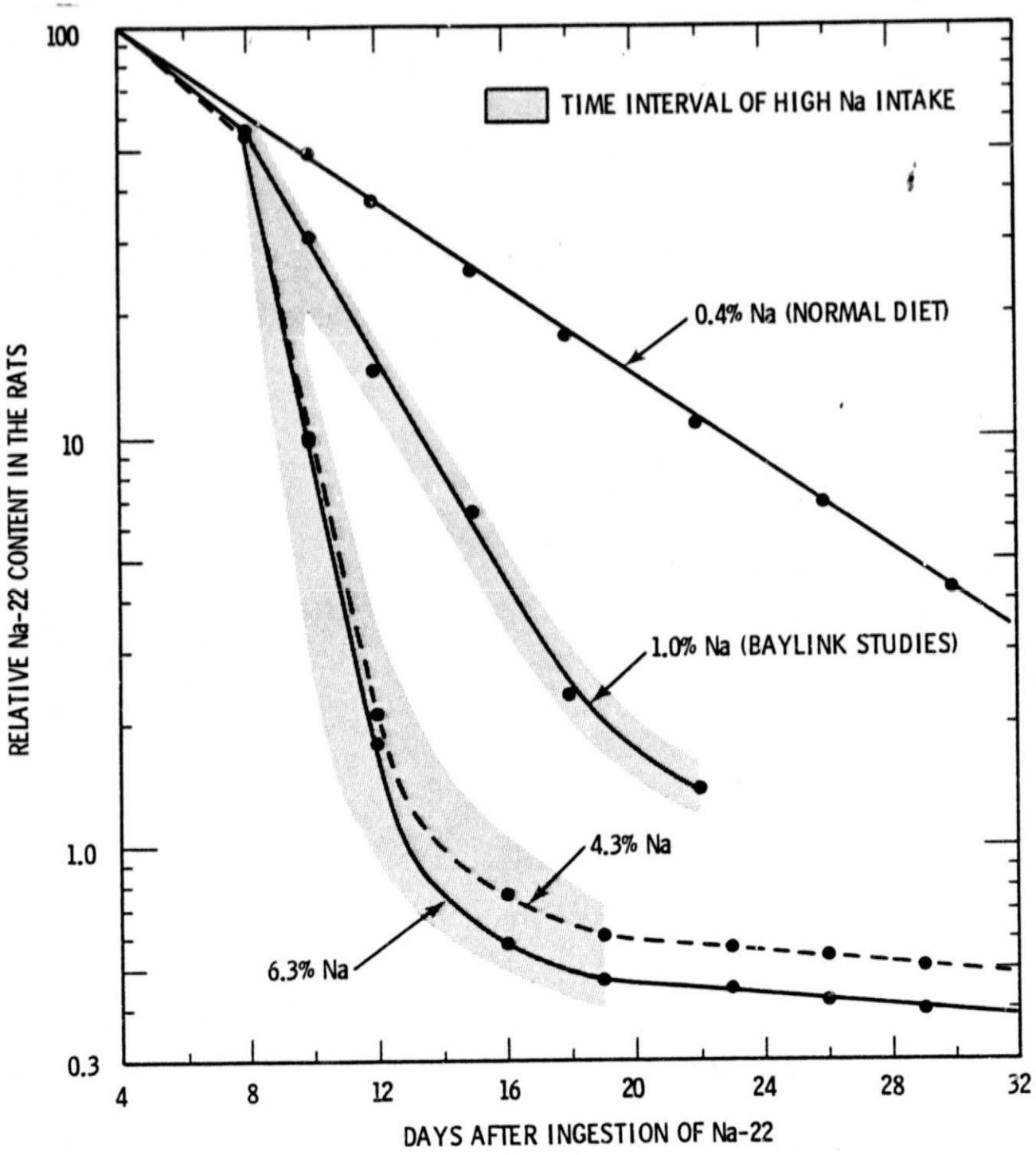

FIGURE 5. Retention of ^{22}Na in Rats Eating Food Containing High NaCl

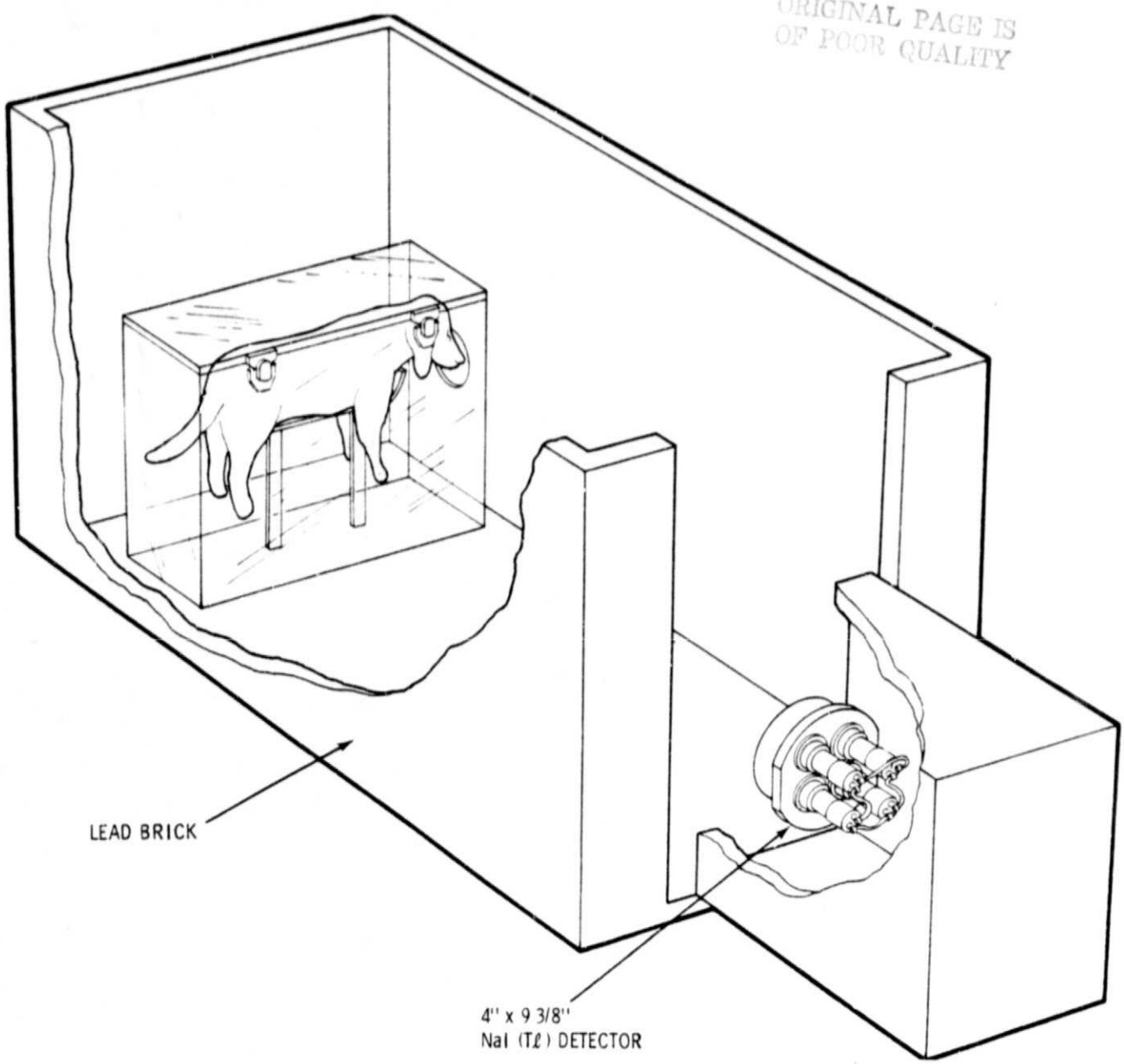

FIGURE 6. Dog Whole Body Counter

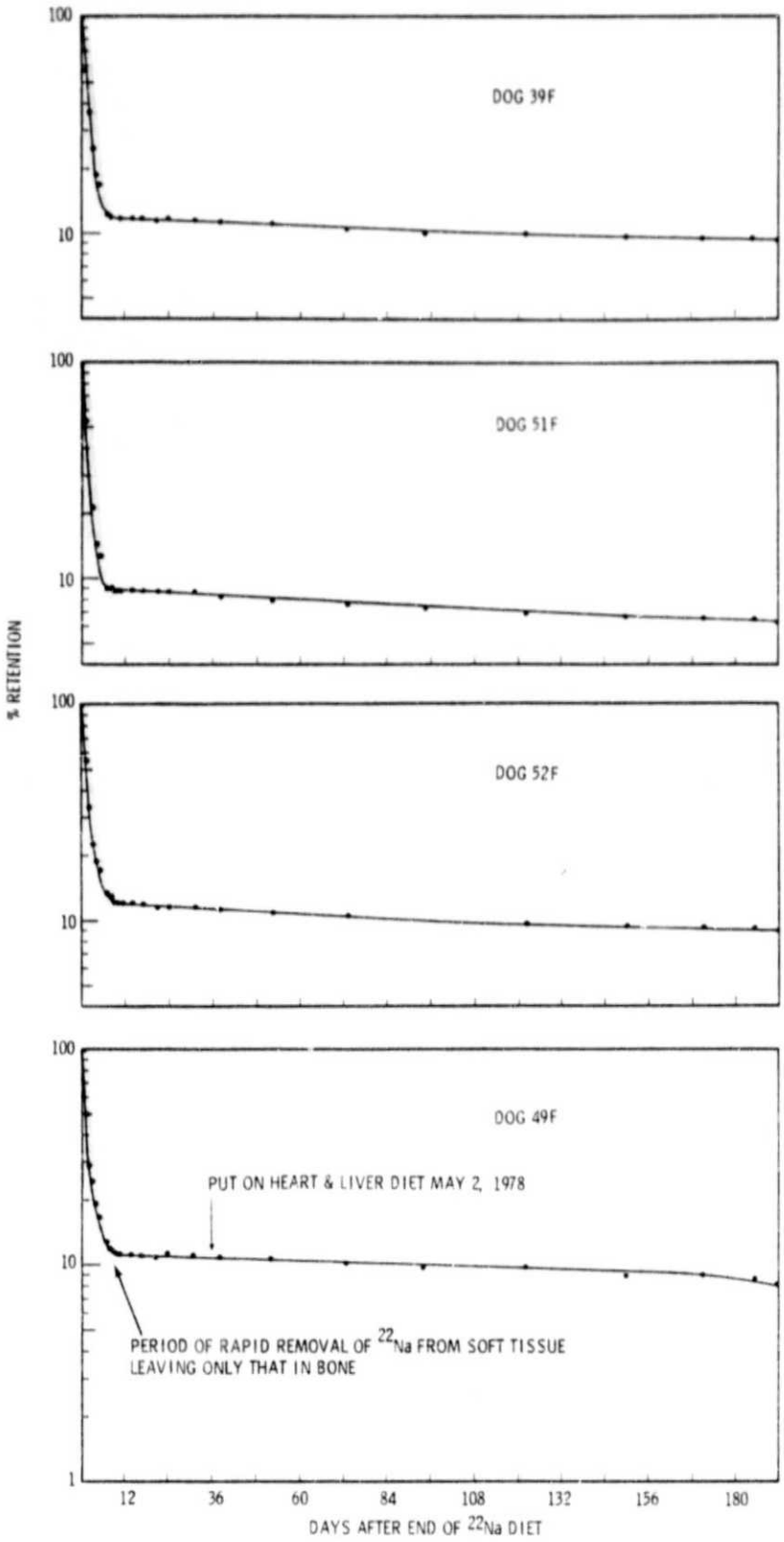

FIGURE 7. Retention of ^{22}Na in Dog Bone

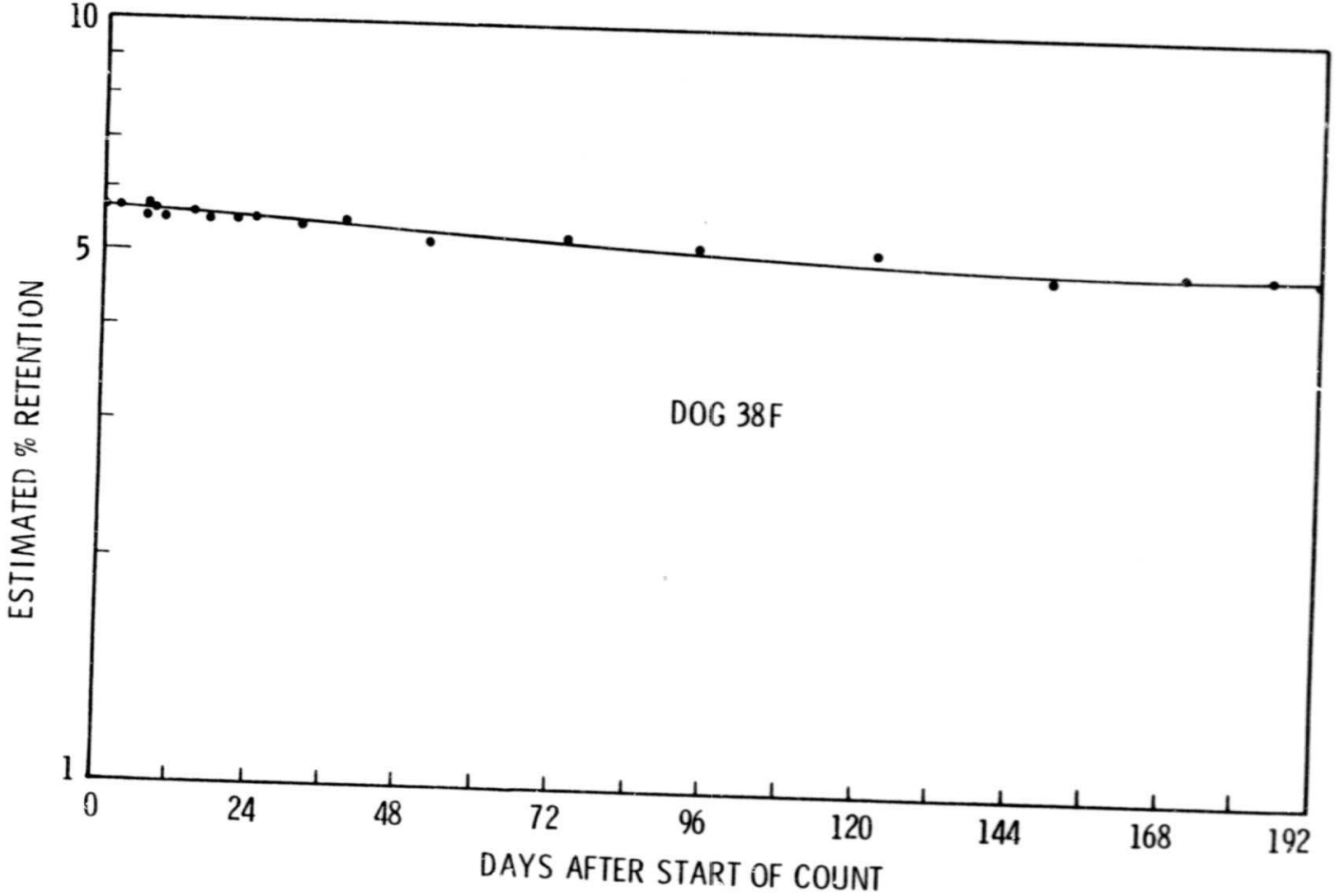

FIGURE 8. Retention of ^{22}Na in Dog 38F

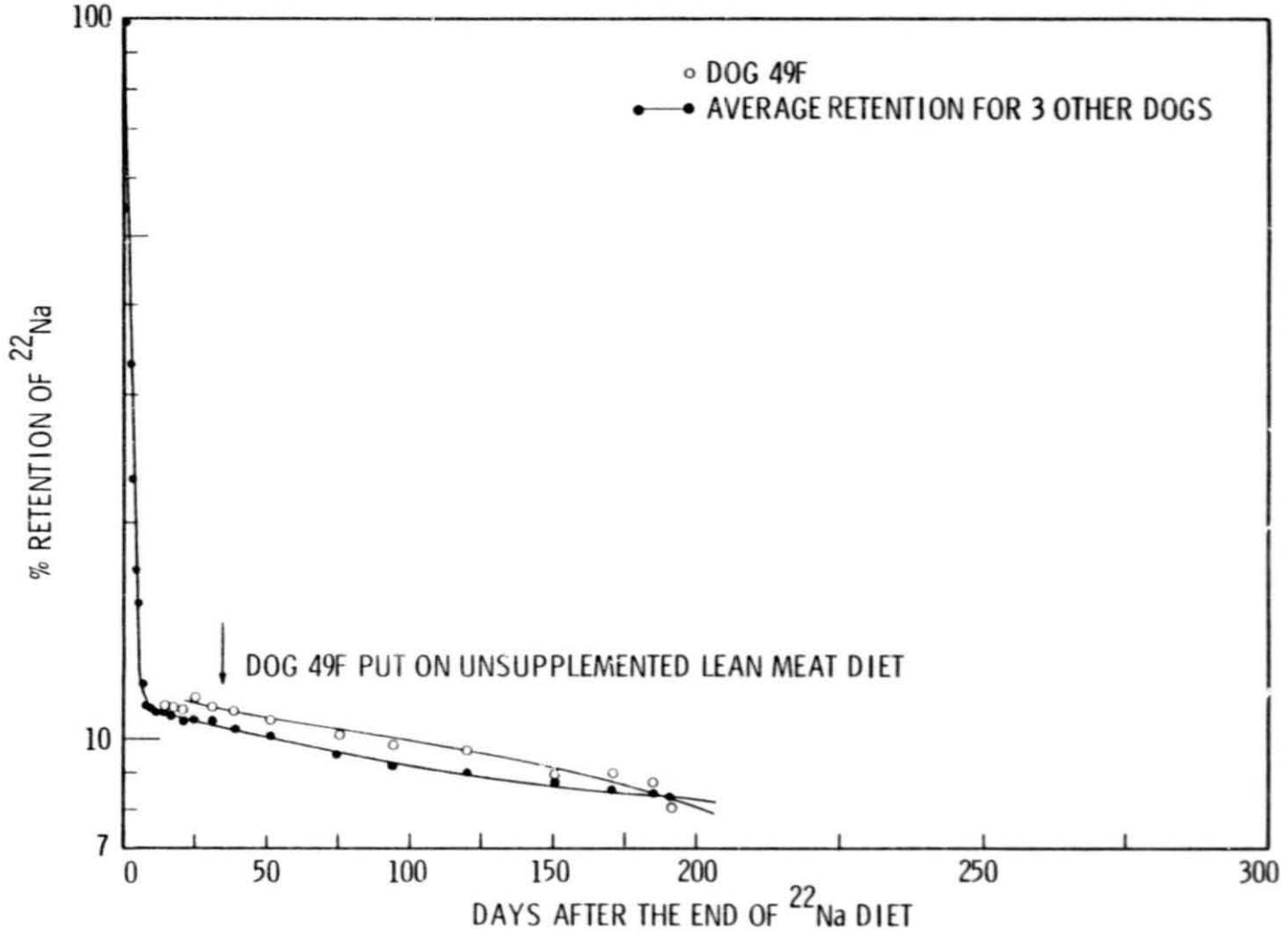

FIGURE 9. Comparison of ^{22}Na Retention in Dogs on Lean Meat and Normal Diet